REPTILES & AMPHIBIANS

The Chin Woodfrog, *Sylvirana lacrima* Sheridan and Stuart 2018 (Amphibia: Anura: Ranidae), from Itanagar Wildlife Sanctuary, a New State Record for Arunachal Pradesh, India

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The anuran genus *Sylvirana* Dubois 1992, with 12 known species, is endemic to southeastern Asia and the Indian Subcontinent (Frost 2023), where only two species, *S. nigrovittata* (Blyth 1856) and *S. lacrima* Sheridan and Stuart 2018, are known to occur. In India, *S. nigrovittata* has been reported from Assam, Arunachal Pradesh, Meghalaya, and West Bengal (Frost 2022), whereas *S. lacrima* has been recorded only from the Dampa Tiger Reserve in Mizoram (Decemson et al. 2021) and a report from Manipur (Decemson et al. 2022). Lalronunga et al. (2021) implied that all records of *Indosylvirana nicobariensis* from India and Bangladesh are referrable to this species, although confirmation is required. Although *Indosylvirana nicobariensis* is reported to occur in the Himalayan state of Arunachal Pradesh (Sarkar and Ray 2006; Mathew and Sen 2010), it needs validation because of close clustering of its genomic sequences with the holotype of *S. lacrima* (Lalronunga et al. 2021). Based on morphological as well as molecular data, we herein report the occurrence of *S. lacrima* in Arunachal Pradesh for the first time.

We used Visual Encounter Surveys (VES) (Crump and Scott 1994) for surveying and a *Sylvirana* sp. was collected in a funnel net. Morphological measurements were taken following Sheridan and Stuart (2018). The specimen was fixed in 10% buffered formalin and before being transferred to 70% ethanol. Prior to fixation, a liver tissue sample was excised and preserved in absolute ethanol for DNA extraction. The whole genomic DNA was extracted from the tissue samples using QIAamp DNA Mini Kit (Cat No.ID: 51306, Qiagen, Valencia, California, USA) following the manufacturer's protocol. A ~580 bp fragment of mitochondrial 16S rRNA gene was amplified and sequenced using forward (L02510- CGC CTG TTT ATC AAA AAC AT) (Palumbi 1996) and reverse primers (H03063-CTC CGG TTT GAA CTC AGA TC) (Rassmann 1997). The PCR thermal regime for amplification was 5 min at 95 °C for initial denaturation, followed by 35 cycles of 1 min at 95 °C for denaturation, 30 sec for annealing at 50.3 °C, elongation for 1 min at 72 °C, and a final elongation for 5 min at 72 °C. The amplicons were checked on 1.5% agarose gel containing ethidium bromide.

In order to confirm the identity of our samples through DNA barcoding, we retrieved the 16S rRNA gene conge-

Figure 1. An adult female *Sylvirana lacrima* from the Senkili Bosti, Lower Chimpu, Itanagar Wildlife Sanctuary, Papumpare District, Arunachal Pradesh, India. Photographs by Kirty Prosad Nath.

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ner's sequences of *Sylvirana* spp. from the NCBI Genbank. A sample of *Bufoides meghalayanus* (MZMU2091) was used as the outgroup. All sequences were aligned using the MUSCLE algorithm in MEGA-X (Kumar et al. 2018). A Maximum Likelihood phylogenetic tree was constructed by executing 1,000 rapid bootstrap replicates with ML search using the GTR+G model in raxmlGUI 2 (Silvestro and Michalak 2012). Uncorrected pairwise genetic distances were calculated in MEGA-X (Kumar et al. 2018). The newly generated ML tree was viewed and edited using Figtree 1.4.4. The specimen was deposited in the National Zoological Collection of the Zoological Survey of India (ZSI), Arunachal Pradesh Regional Centre (APRC), bearing the registration number V/APRC/A-313. The aerial distances between different localities are calculated using Google Earth Pro.

A female *S. lacrima* (Fig. 1) (SVL 46.43 mm) was collected from the Senkili Bosti, Lower Chimpu, Itanagar Wildlife Sanctuary, Papumpare District, Arunachal Pradesh, India (27.065 N, 93.634 E; elev. 187 m asl) at 1342 h 18 August 2020. This record extends the distribution 613 km northwest of the type locality of *S. lacrima* in Mindat, Myanmar and 413 km north of the closest known population in the Dampa Tiger Reserve in Mizoram. The frog was collected from the mouth of a hill stream containing slippery rocks with mosses and vegetation and surrounded by ferns, cardamom, and other shrubs. Lalremsanga (2011) reported that breeding occurs during the months of October to February in Mizoram. The males call mainly during the night beginning at 1630 h and unsuccessful males continue until the next morning. The call consisted of 5–9 low pitched notes that sound like faint crying along streams and other bodies of water like backwaters, drains, tanks, and littoral undergrowth of ponds and lakes, where breeding and oviposition take place.

The morphology of the frog (Table 1) matched the diagnostic features of *S. lacrima* (Sheridan and Stuart 2018). Also, our sample (ON493539) from Arunachal Pradesh was nested with *S. lacrima* (MG935997) from the type locality in Mindat (Myanmar) and other known populations of the species from Manipur (OM574589) (communicated in separate article) and Mizoram (MW076232), with genetic distances of 0.00–0.01% (Table 2). The ML phylogeny tree (Fig. 2)

Table 1. Measurements (in mm) of *Sylvirana lacrima* from Itanagar WLS, Arunachal Pradesh, India. Abbreviations: SVL = snout-vent length; HL = head length; HW = head width; MN = mandible-nostril distance; TYD = tympanum diameter; TYE = tympanum-eye distance; EL = eye length; EN = eye-nostril distance; SN = snout-nostril distance; SL = snout to eye distance; IFE = distance between the front of eyes; IBE = distance between the back of eyes; FLL = forelimb length; HAL = hand length; TFL = third finger length; FL = femur length: TL = tibia length; TFOL = tarsus-foot length; FTL = fourth toe length; ITL = inner toe length).

Parameters	Measurements (mm)
SVL	46.43
HL	20.09
HW	14.79
MN	17.76
TYD	3.68
TYE	1.68
EL	6.09
EN	4.9
SN	3.39
SL	8.19
IFE	6.04
IBE	9.76
FLL	10.36
HAL	9.04
TFL	6.08
FL	20.76
TL	25.39
TFOL	16.8
FTL	9.47
ITL	4.39

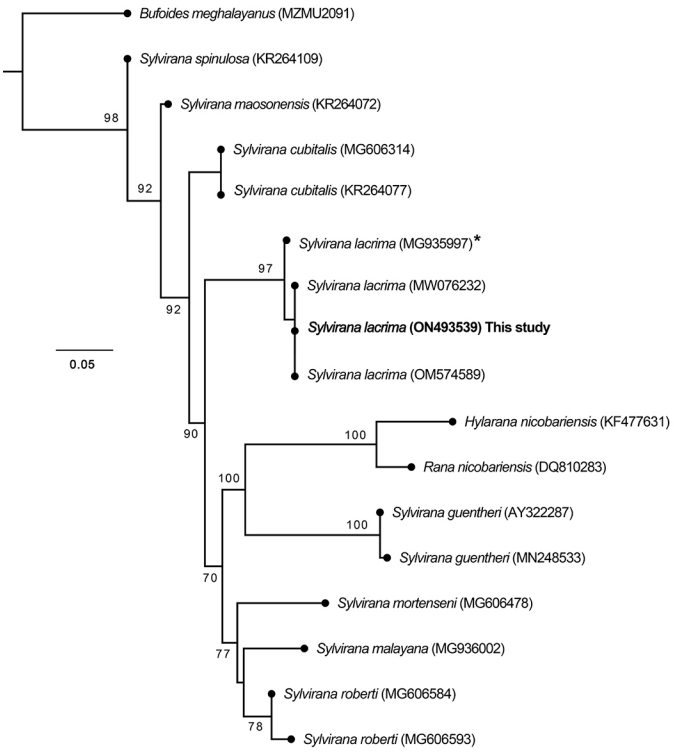

Figure 2. Maximum Likelihood phylogenetic tree based on the 16S rRNA sequences confirming the genetic identity of *Sylvirana lacrima* from Arunachal Pradesh. Numbers at nodes indicate Bayesian posterior probability (BPP). BOLD lettering represents our study specimen and the asterisk (*) denotes the type locality, followed by the NCBI accession number.

Table 2. Uncorrected k2p distance of *Sylvirana* spp. through 16S rRNA partial gene sequences.

SNo.	Taxa	K2p distance															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	*Sylvirana lacrima*_ON493539																
2	*Sylvirana lacrima*_MG935997*	0.01															
3	*Sylvirana lacrima*_OM574589	0.00	0.01														
4	*Sylvirana lacrima*_MW076232	0.00	0.01	0.00													
5	*Sylvirana malayana*_MG936002	0.08	0.08	0.08	0.08												
6	*Sylvirana guentheri*_MN248533	0.09	0.09	0.09	0.09	0.09											
7	*Sylvirana guentheri*_AY322287	0.08	0.08	0.08	0.08	0.09	0.01										
8	*Sylvirana mortenseni*_MG606478	0.09	0.10	0.09	0.09	0.09	0.11	0.11									
9	*Sylvirana roberti*_MG606593	0.09	0.09	0.09	0.09	0.06	0.10	0.10	0.06								
10	*Sylvirana roberti*_MG606584	0.07	0.08	0.07	0.07	0.06	0.09	0.09	0.06	0.01							
11	*Sylvirana maosonensis*_KR264072	0.05	0.05	0.05	0.05	0.07	0.07	0.06	0.07	0.06	0.05						
12	*Sylvirana spinulosa*_KR264109	0.06	0.06	0.06	0.06	0.09	0.07	0.07	0.08	0.08	0.07	0.02					
13	*Sylvirana cubitalis*_MG606314	0.07	0.07	0.07	0.07	0.09	0.07	0.07	0.07	0.07	0.06	0.02	0.03				
14	*Sylvirana cubitalis*_KR264077	0.07	0.07	0.07	0.07	0.09	0.07	0.07	0.07	0.07	0.06	0.02	0.03	0.00			
15	*Sylvirana nicobariensis*_KF477631	0.09	0.09	0.09	0.09	0.10	0.10	0.10	0.10	0.09	0.09	0.08	0.07	0.09	0.09		
16	*Sylvirana nicobariensis*_DQ810283	0.09	0.10	0.09	0.09	0.10	0.10	0.10	0.10	0.09	0.09	0.09	0.08	0.09	0.09	0.02	
17	*Bufoides meghalayanus*_MZMU2091	0.12	0.12	0.12	0.12	0.12	0.11	0.10	0.14	0.15	0.15	0.11	0.11	0.10	0.10	0.14	0.14

established high support for clustering of all populations of *S. lacrima*. The observed interspecific divergence of *Sylvirana* spp. was 0.00–9.00%.

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